

From the Department of Dermatology, University of Lund, Sweden.
Head: Professor G. Hagerman, M.D.

BASOPHILS AND OTHER LEUCOCYTES IN ALLERGIC REACTIONS IN GUINEA-PIGS

By

HANS RORSMAN

Two cell-types with metachromatic granules are present in the guinea-pig—the tissue mast cells and the basophil leucocytes. The tissue mast cells of the guinea-pig show degranulation in anaphylaxis (*Stuart* 1952; *Mota & Vugman* 1956, *Boréus* 1960) and addition of antigen to sensitized guinea-pig tissue *in vitro* also causes mast cell degranulation (*Mota* 1959; *Humphrey & Mota* 1959; *Boréus & Chakravarty* 1960). Mast cell degranulation is combined with release of histamine and of 'slow reacting substance'—'SRS' (*Boréus & Chakravarty* 1960). There is thus strong evidence that mast cells play a part in guinea-pig anaphylaxis. Our knowledge of the relation of the basophil leucocytes to allergic reactions in guinea-pigs is however very limited (*Levaditi* 1902; *Schlecht* 1910; *Ringo* 1923; *Plimpton* 1940; *Winqvist* 1960; *Chan & Yoffey* 1960).

The present study deals with the reactions of basophils in anaphylactic conditions in guinea-pigs. Thus basophils in circulating blood are studied in connection with:

1. Fatal anaphylaxis induced by intravenous injection of a large dose (1 mg) of allergen.
2. Acute mild anaphylactic reaction induced by intravenous injection of a small dose (0.01 mg) of antigen.
3. Prolonged anaphylaxis, induced by subcutaneous injection of a sub-lethal dose (5 mg) of allergen.

Neutrophils, lymphocytes, and eosinophils are studied simul-

taneously in order to observe possible co-reactions of basophils and other leucocytes.

MATERIAL AND METHODS

Male guinea-pigs on a diet consisting of hay and beets, weighing 250–440 g, were used.

The *antigen* used was crystalline egg albumin (Kebo AB, Stockholm). All solutions were prepared in saline and sterilized by passage through a Seitz filter.

The volume of all injections was 1 ml. Physiologic saline was used for preparation of solutions.

Sensitization was induced by a subcutaneous injection of 100 mg of egg albumin followed 3–4 days later by the same dose intraperitoneally.

Challenging injections were made three to four weeks after the first sensitizing injection.

Intravenous injections were made into the upper surface of the hind feet using 26 gauge needles according to the technique described by *Kabat & Mayer* (1948).

Subcutaneous injections were made into the skin of the dorsum just caudally to the ribs using 26 gauge needles.

Samples for blood cell counts were obtained after incision of an ear vein. The skin was previously cleaned with ethyl-ether. The first blood-drop was not used. Counts were made 15 minutes and immediately before challenging injections and then 7 minutes, 15 minutes, 1 hour, 4 hours, and 24 hours after the injection. In the experiment with fatal anaphylaxis and in its control experiment the chest was opened immediately after death and blood was aspirated into a syringe from the right ventricle.

The *hematological technique* was the same as that used in man by *Rorsman & Rosengren* (1958). Eosinophils were, however, counted in the same dilution fluid as basophils. Basophils and eosinophils were easily identified with the chamber method used. The granules of guinea-pig basophils are larger and darker than those in human basophils. The eosinophil

granules are greenish-grey. For further description of guinea-pig blood morphology, cf. *Schermer* (1954).

The counts of neutrophils and lymphocytes were calculated from total leucocyte counts and differential counts of 100 leucocytes.

Absolute counts of basophils were compared with the indirect calculation of basophils from total leucocyte counts and differential counts in 100 cases. The mean values of the direct and the indirect determination were the same.

The following experiments were performed:

- A. Egg albumin (1 mg) was injected intravenously in five sensitized guinea-pigs.
- B. As a control for experiment A egg albumin (1 mg) was injected intravenously in five unsensitized animals. These were killed by intravenous injection of 1 ml air 2-3 minutes after the albumin injection and the postmortal sample was then obtained from the right ventricle as in the animals which had died in anaphylaxis.
- C. Albumin (0.01 mg) was injected intravenously in ten sensitized guinea-pigs.
- D. As a control for experiment C egg albumin (0.01 mg) was injected intravenously in ten unsensitized animals.
- E. Egg albumin (5 mg) was injected subcutaneously in ten sensitized guinea-pigs.
- F. As a control for experiment E egg albumin (5 mg) was injected subcutaneously in ten unsensitized guinea-pigs.

RESULTS

Symptoms.

The *intravenous injection of 1 mg egg albumin* in the *sensitized* animals induced anaphylactic death 3-7 minutes after the injection in all animals. The *intravenous injection of 1 mg egg albumin* in the *unsensitized* did not induce any symptoms but immediately after the following intravenous air-injection severe convulsions occurred and death followed within 1 to 3 minutes. The *intravenous injection of 0.01 mg egg albumin* in

the *sensitized* animals induced sneezing, dyspnoea, ruffled fur and sometimes urination and defecation. The symptoms subsided within 5–15 minutes. Blood sampling was difficult in some animals while symptoms lasted since very little blood appeared after nicking. The *intravenous injection of 0.01 mg* egg albumin induced no symptoms in the *unsensitized* animals.

After *subcutaneous injection of 5 mg* albumin in the *sensitized* animals these became weak after a few minutes and remained so for more than 4 hours. Respiratory symptoms were never pronounced. After 24 hours the animals appeared normal. Blood sampling was difficult 1 and 4 hours after the injection as very little blood appeared after nicking. *Subcutaneous injection of 5 mg* egg albumin did not induce any symptoms in the *unsensitized* animals.

Effects on blood cells.

Variance analysis was used in the statistical treatment. However, changes of mean values were always judged on the basis of individual changes, because of different magnitudes of the variation within the animals and because of the risk of correlation between values that were chronologically close together.

Intravenous injection of 1 mg egg albumin.

Mean values of the pre-injection counts, obtained from the peripheral blood, and of the postmortal counts, obtained from the right ventricle, are given in Table 1 for *sensitized* animals and in Table 2 for the *unsensitized* ones.

In *sensitized* animals the ranges of the pre-injection values were for basophils 39–204, for neutrophils 3,900–10,500, for lymphocytes 2,200–7,400, and for eosinophils 32–142 cells per mm³.

In *unsensitized* animals the ranges of pre-injection values were for basophils 20–100, for neutrophils 2,800–4,500, for lymphocytes 1,500–4,100, and for eosinophils 25–70 cells per mm³.

In the *sensitized* animals the basophils were postmortally

TABLE 1

The effect on leucocytes of intravenous injection of 1 mg egg albumin in sensitized guinea-pigs. Mean values of determinations in five animals are given.

	15 min. before injection	Immediately before injection	Immediately after death
Basophils cells/mm ³	87	93	0
Neutrophils 1000 cells/mm ³	6.9	7.6	0.9
Lymphocytes 1000 cells/mm ³	4.5	5.1	3.6
Eosinophils cells/mm ³	79	74	13

TABLE 2

The effect on leucocytes of intravenous injection of 1 mg egg albumin in unsensitized guinea-pigs. Mean values of determinations in five animals are given.

	15 min. before injection	Immediately before injection	Immediately after death
Basophils cells/mm ³	44	47	46
Neutrophils 1000 cells/mm ³	3.8	3.6	2.7
Lymphocytes 1000 cells/mm ³	2.4	2.6	4.1
Eosinophils cells/mm ³	41	40	41

decreased to a zero-level. Also the other granulocytes were considerably decreased, while the lymphocytes were slightly affected. Decrease of basophils, neutrophils, and eosinophils was pronounced in all five animals, while the lymphocytes decreased in four out of the five animals studied. In the *unsensitized* animals no definite cell changes occurred.

Intravenous injection of 0.01 mg egg albumin.

The mean values of cell counts in *sensitized* animals are given in Table 3.

The mean values and standard deviations of pre-injection counts were for basophils 91 ± 55 , for neutrophils $4,400 \pm 1,700$, for lymphocytes $6,800 \pm 2,600$, and for eosinophils 107 ± 52 cells per mm³.

Seven minutes after the intravenous injection of albumin in

TABLE 3

The effect on leucocytes of intravenous injection of 0.01 mg egg albumin in sensitized guinea-pigs. Mean values of determinations in ten animals are given. Figures in brackets indicate the number of missing determinations due to technical difficulties.

	Before injection		After injection				
	15'	0	7'	15'	1 h	4 h	24 h
Basophils							
cells/mm ³	89	92(1)	17(1)	22	55	53	92
Neutrophils							
1000 cells/mm ³	4.3	4.5	4.5(1)	4.3	9.4	7.8	4.8
Lymphocytes							
1000 cells/mm ³	7.4	6.3	6.3(1)	6.6	6.9	5.6	6.1
Eosinophils							
cells/mm ³	113	100(1)	123(1)	176	183	270	161

TABLE 4

The effect on leucocytes of intravenous injection of 0.01 mg egg albumin in unsensitized guinea-pigs. Mean values of determinations in ten animals are given.

	Before injection		After injection				
	15'	0	7'	15'	1 h	4 h	24 h
Basophils							
cells/mm ³	41	46	51	52	54	46	50
Neutrophils							
1000 cells/mm ³	4.6	4.7	4.8	5.2	6.0	7.3	5.5
Lymphocytes							
1000 cells/mm ³	4.5	4.6	4.4	4.1	3.6	3.0	3.9
Eosinophils							
cells/mm ³	50	48	67	68	86	43	49

the *sensitized* guinea-pigs the *basophil* mean value was markedly lowered and a decrease compared with the individual mean values of the pre-injection determinations was found in nine out of the ten animals. The mean value was at the same low level after 15 minutes and all ten animals now showed

values below the pre-injection counts. The relatively low mean values 1 and 4 hours after the injection were due to counts lower than the pre-injection ones in eight and nine animals respectively. After 24 hours the pre-injection mean value was reached.

The *neutrophils* did not react immediately after the injection, but after 1 and 4 hours the individual counts were in all animals higher than the pre-injection counts and the mean values were considerably increased. Neutrophils returned to the pre-injection level after 24 hours.

There was no significant change of the *lymphocytes*.

The higher mean values of the *eosinophils* after the injection were due to a rise in all ten animals after 15 minutes and in eight out of ten animals after 4 hours, while the increased mean values on the other occasions were due to increase in a less significant number of animals.

The effect of 0.01 mg albumin injected intravenously in the *unsensitized* animals appears from Table 4.

The mean values and standard deviations of pre-injection counts were for basophils 44 ± 25 , for neutrophils $4,600 \pm 2,500$, for lymphocytes $4,500 \pm 2,600$, and for eosinophils 49 ± 29 cells per mm^3 .

Basophils were not influenced.

Neutrophil mean values were higher 1 and 4 hours after the injection than before, and the increased mean values were due to a neutrophil rise in eight out of the ten animals studied.

Lymphocytes showed slightly depressed mean values after the injection and the individual counts were decreased compared with the pre-injection counts in eight out of the ten animals after 4 and 24 hours.

Eosinophils did not show any uniform changes after the injection, although increases of mean values were observed.

Subcutaneous injection of 5 mg egg albumin.

Mean values of cell counts in *sensitized* animals are given in Table 5.

The mean values and standard deviations of pre-injection counts were for basophils 71 ± 41 , for neutrophils $4,900 \pm 1,900$, for lymphocytes $5,700 \pm 2,300$, and for eosinophils 67 ± 33 cells per mm^3 .

In sensitized animals the subcutaneous injection of 5 mg egg albumin induced pronounced changes in the *basophil* cell count.

TABLE 5

The effect on leucocytes of subcutaneous injection of 5 mg egg albumin in sensitized guinea-pigs. Mean values of determinations in ten animals are given. Figures in brackets indicate the number of missing determinations due to technical difficulties.

	Before injection		After injection				
	15'	0	7'	15'	1 h	4 h	24 h
Basophils cells/ mm^3	68	74	44	13	43(3)	17(1)	120
Neutrophils 1000 cells/ mm^3 .	4.6	5.2	4.9	4.8	8.9(3)	15.6(2)	4.7
Lymphocytes 1000 cells/ mm^3 .	5.5	5.8	5.1	5.1	5.1(3)	7.9(2)	6.0
Eosinophils cells/ mm^3	61	73	85	72	365(3)	346(1)	475

TABLE 6

The effect on leucocytes of subcutaneous injection of 5 mg egg albumin in unsensitized guinea-pigs. Mean values of determinations in ten animals are given.

	Before injection		After injection				
	15'	0	7'	15'	1 h	4 h	24 h
Basophils cells/ mm^3	63	56	76	67	76	82	114
Neutrophils 1000 cells/ mm^3	5.9	5.3	5.8	6.2	8.4	8.9	6.3
Lymphocytes 1000 cells/ mm^3	5.1	4.3	5.0	4.8	5.0	4.7	6.0
Eosinophils cells/ mm^3	50	49	54	48	57	49	50

The low value after 7 minutes was due to a decrease of the basophil counts compared with the pre-injection counts in eight out of the ten animals, while the low mean value after 15 minutes was due to decreases in all the studied animals. The lower mean value 1 hour after the injection was due to pronouncedly low values in five animals. The more pronounced decrease after 4 hours was due to a decrease in nine animals. The basophil level after 24 hours was higher than the pre-injection counts in eight out of the ten animals.

Mean values of *neutrophils* were increased after 1 and 4 hours compared with pre-injection counts. The individual values were increased except in one animal. The very high mean value after 4 hours was partly due to pronounced increase in two animals.

Lymphocytes did not show significant changes.

Eosinophils were considerably increased 1, 4, and 24 hours after the injection. At these times the eosinophils were above pre-injection levels in all the studied animals.

The mean values of cell counts in *unsensitized* animals are given in Table 6.

The mean values and standard deviations of pre-injection counts were for basophils 60 ± 40 , for neutrophils $5,600 \pm 3,300$, for lymphocytes $4,700 \pm 1,900$, and for eosinophils 49 ± 33 .

Unsensitized animals did not show any immediate reaction of *basophils*, unlike the sensitized animals, but after 24 hours the mean value was considerably increased owing to increases from pre-injection counts in nine out of the studied animals.

The increased mean values of *neutrophils* 1 and 4 hours after the injection were due to increases above pre-injection counts in nine of the ten animals.

Lymphocytes and *eosinophils* did not change significantly.

DISCUSSION

The values of the different leucocytes in untreated animals agree with those given in literature (Scherner 1954; Back, Feinman & Harrisson 1955; Winqvist 1960).

The pronounced decrease of *granulocytes* during anaphylactic shock, observed in the actual experiments, is well known through earlier work (*Weiss & Tsuru* 1910; *Wittkower* 1923; *Bickel & Frommel* 1924). It may be observed that the decrease of the basophils seemed to be still more pronounced than that of the other granulocytes.

By intravenous injection of a lower dose of antigen (0.01 mg), which did not induce fatal anaphylaxis, a dissociation of the cellular reaction could be obtained so that the animals showed a decrease of *basophils* without a concomitant decrease of the other leucocytes. The decrease after the antigen injection was obvious after 7 and 15 minutes, but thereafter a return toward pre-injection values could be observed.

Also after the subcutaneous injection of 5 mg egg albumin in sensitized animals the *basophils* showed a rapid decrease, most pronounced after 15 minutes. After subcutaneous injection the return toward normal levels was interrupted by a new decrease after 4 hours. This decrease of basophils after 4 hours was not observed after the intravenous injection of 0.01 mg of albumin. After 24 hours the basophils were above the initial count, in contrast to the condition after the intravenous injection of 0.01 mg. However, basophils were increased 24 hours after subcutaneous injection of 5 mg egg albumin also in unsensitized animals.

The *neutrophils* decreased in the fatal anaphylaxis but in experiments with non-fatal anaphylaxis no decrease was found. It should be noted that the neutrophils in all experiments showed higher mean values 1 and 4 hours after the injections than before, irrespectively of the immunologic state of the animal.

Changes of the *lymphocytes* were not pronounced in any of the experiments.

Eosinophils decreased like the other granulocytes during the fatal anaphylaxis. However, the eosinophils did not decrease during mild acute or prolonged anaphylaxis like the basophils. Instead an eosinophilia was induced, which was pronounced for the 5 mg dose. The post-anaphylactic eosinophilia, which

has been known for a long time (*Schlecht* 1910) has been extensively studied (cf. *Samter* 1949). Liberated histamine may possibly play a rôle in the induction of anaphylactic eosinophilia (*Back et al.* 1955).

During sensitization of guinea-pigs by repeated injections of a foreign protein the eosinophils and basophils increase concomitantly (*Winqvist* 1960). The actual findings of a basophil decrease without fall of other blood cells, eosinophils included, indicate a mutual *independence of the basophils and eosinophils*.

The mechanism for the basophil decrease in anaphylaxis has not yet been investigated. It seems possible that the cells are degranulated like tissue mast cells in anaphylaxis (*Mota & Vugman* 1956; *Boréus* 1960). Degranulated basophils would probably be impossible to diagnose. A total destruction or a selective trapping of the basophils in some organ during anaphylaxis could also explain the basopenia.

Nothing is known about the agent responsible for the basopenia, in whatever way it may be induced.—The allergen might be effective directly but substances released during the anaphylaxis should also be considered.

Selective basopenia has earlier been demonstrated in certain forms of human urticaria (*Rorsman & Rosengren* 1958; *Meneghini & Levi* 1959). In rabbits it has not been possible to induce selective basopenia (*Rorsman* in press). The present finding of basopenia without decrease of other leucocytes in guinea-pig anaphylaxis will form a further basis for studies on the functional importance of basophil leucocytes.

SUMMARY

Basophils and other leucocytes were studied in fatal, in mild acute, and in moderately severe prolonged anaphylaxis in guinea-pigs.

It was found that:

1. All granulocytes decreased in fatal anaphylaxis.
2. Basophils decreased selectively in mild acute and in moderately severe prolonged anaphylaxis.

3. Lymphocytes and neutrophils did not change specifically in mild acute or in moderately severe prolonged anaphylaxis.
4. Eosinophils increased irregularly after mild acute but regularly during moderately severe prolonged anaphylaxis.

ACKNOWLEDGEMENTS

My thanks are due to Mrs. *E. Wiehe* and Mrs. *H. Rämmal* for skilful technical assistance and to Professor *C.-E. Quensel*, who has given me advice and performed the statistical analysis.

REFERENCES

- Back, N., Feinman, J. I. & Harrison, J. W. E.*: Effect of antihistamines and histamine desensitization on hematologic changes induced in guinea pigs by histamine. *Acta allergol.* 8, 112, 1955.
- Bickel, G. & Frommel, E.*: La repartition des globules blancs dans le choc anaphylactique experimental. *J. physiol. path. gén.* 22, 625, 1924.
- Boréus, L. O.*: Quantitative studies on the anaphylactic mast-cell reaction in vivo in the guinea pig. *Acta physiol. scand.* 48, 431, 1960.
- Boréus, L. O. & Chakravarty, N.*: Tissue mast cells, histamine and slow reacting substance in anaphylactic reaction in the guinea pig. *Acta physiol. scand.* 48, 315, 1960.
- Chan, B. S. T. & Yoffey, J. M.*: The basophil cells of guinea-pig bone marrow and their response to horse serum. *Immunology.* 3, 237, 1960.
- Humphrey, J. H. & Mota, I.*: The mechanism of anaphylaxis. Specificity of antigen—induced mast cell damage in anaphylaxis in the guinea pig. *Immunology.* 2, 31, 1959.
- Kabat, E. A. & Mayer, M. M.*: Experimental immunochemistry. Springfield, Thomas 1948, p. 546.
- Levaditi, C.*: Contribution à l'étude des mastzellen et de la mastzellenleucocytose. Thesis, Paris, Naud, 1902.
- Meneghini, C. L. & Levi, L.*: Istaminemia e mastociti circolanti in alcune dermatosi allergiche. *Gior. ital. dermat.* 100, 449, 1959.
- Mota, I.*: Effect of antigen and octylamine on mast cells and histamine content of sensitized guinea-pig tissues. *J. Physiol.* 147, 425, 1959.
- Mota, I. & Vugman, I.*: Effects of anaphylactic shock and compound 48/80 on the mast cells of the guinea pig lung. *Nature.* 177, 427, 1956.
- Plimpton, N. C., Jr.*: Basophil leucocytes and myelocytes after local injection of ventriculin. *Anat. Rec.* 76, 475, 1940.
- Ringoen, A.*: The mast leucocytes in the adult guinea pig under experimental conditions. *Am. J. Anat.* 31, 319, 1923.

- Rorsman, H. & Rosengren, E.:* Basophil leucocytes and blood histamine in urticaria. *Acta dermat.-venereol.* 38, 377, 1958.
- Samter, M.:* The response of eosinophils in the guinea pig to sensitization, anaphylaxis and various drugs. *Blood.* 4, 217, 1949.
- Schermer, S.:* Die Blutmorphologie der Laboratoriumstiere. Leipzig, Johann Ambrosius Barth Verlag, 1954.
- Schlecht, W.:* Über die Einwirkung von Seruminjectionen auf die Eosinophilen und Mastzellen des menschlichen und tierischen Blutes. *Deutsch. Arch. f. klin. Med.* 98, 308, 1910.
- Stuart, E. G.:* Mast cell responses to anaphylaxis. *Anat. Rec.* 112, 394, 1952.
- Weiss, H. & Tsuru:* Ueber den Einfluss des anaphylaktischen Schocks auf das Blut. *Ztschr. Immunitätsf.* 5, 516, 1910.
- Winqvist, G.:* Experimental production of basophil granulocytes in the guinea pig. *Exper. Cell Research.* 19, 7, 1960.
- Wittkower, E.:* Die Veränderungen des Blutes bei der Anaphylaxie. *Ztschr. ges. exper. Med.* 34, 108, 1923.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.